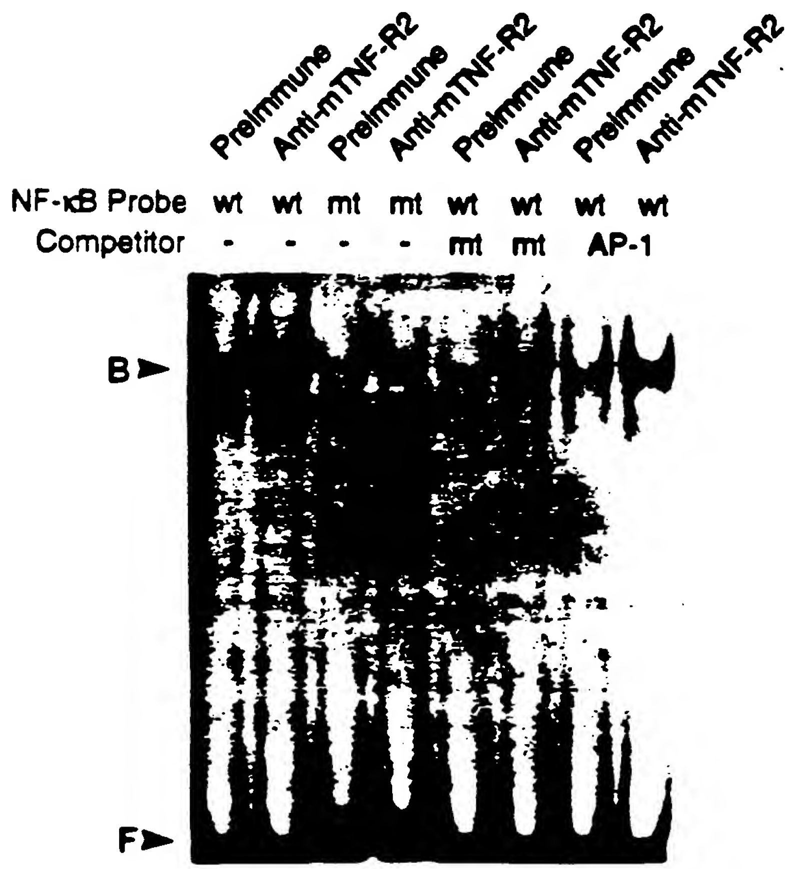
NR 446915

Figure 1

Activation of the Transcription Factor NF-κB through TNF Receptor 2 in CT6 Cells



NR 446915

Figure 2

Immunoprecipitation of Human TNF Receptor 2

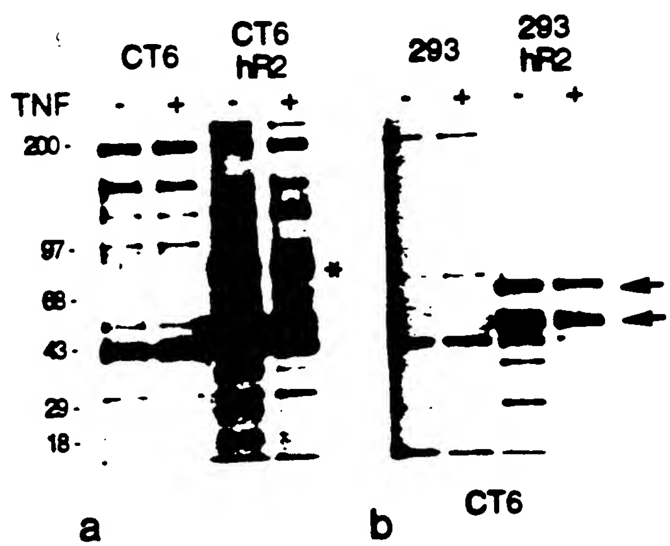
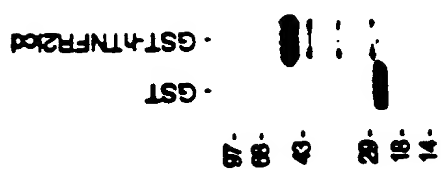


Figure 3



Glutathione-S-Transferase human TNF Receptor 2
Intracellular Domain Fusion Protein

Figure 4

Coprecipitation of Glutathione-S-Transferase
Human TNF Receptor 2 Intracellular Domain
Fusion Protein in CT6 Cell Extracts

Figure 5

Coprecipitation of Glutathione-S-Transferase Mutant Human TNF Receptor 2 Intracellular Domain Fusion Proteins in CT6 Cell Extracts

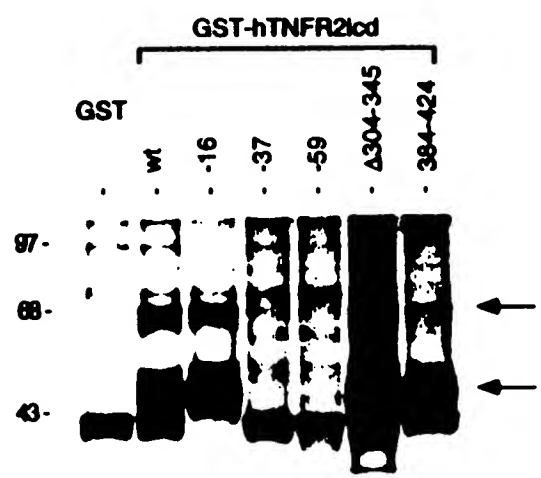


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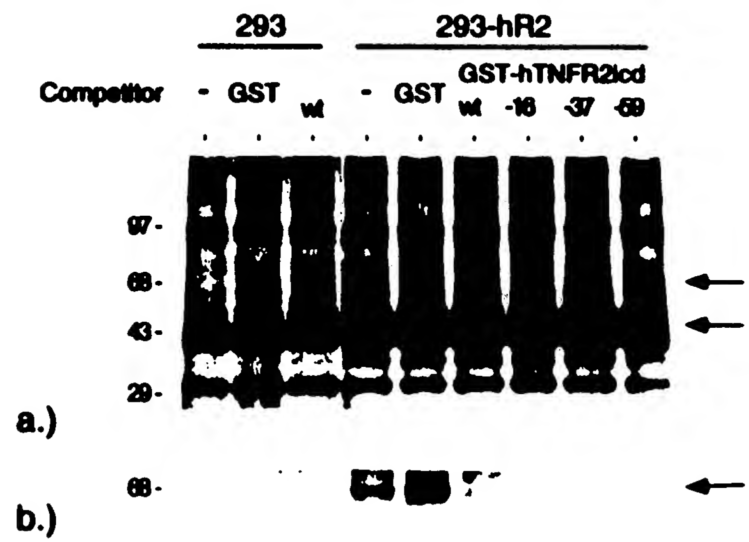
Figure 6

Competition of TNF Receptor 2 Associated Factors with Glutathione-S-Transferase TNF Receptor 2 Intracellular Domain Fusion Proteins



CT6

M 446915

Figure 7

Coprecipitation of Glutathione-S-Transferase Human TNF Receptor 2 Intracellular Domain Fusion Protein in Jurkat Cell Extracts

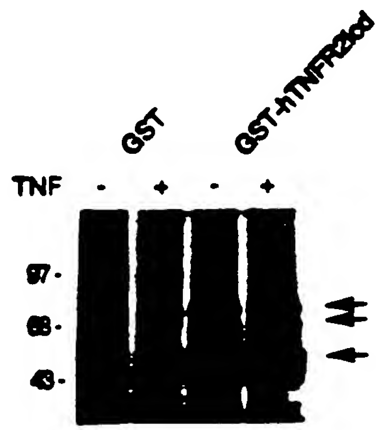


Figure 8

Intracellular Localization of TNF Receptor 2 Associated Factors

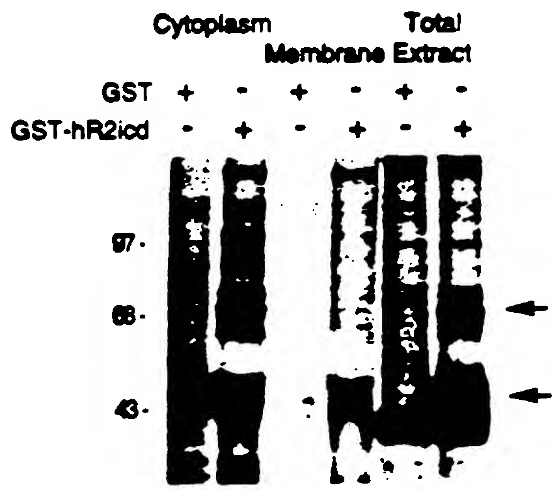


Figure 9

Purification of TNF Receptor 2 Associated Factors

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74 GAGGCTCAGACATATTGAAGACCGTGTGACATAGGGTAGCCAAATGACAGTGTGAGAAAGTGACATTTACTCAAG
149 GCCACCCAGATATCCTGGAGGACCCAGAACCCTGGAGATTCCCATCAGAAAGACCTTCTGGCCACCTGAAACCCC

1 MetAlaSerSerSerAlaProAspGluAsnGluPheGlnPheGlyCysProProAlaProCysGlnAspPro
224 AAGATGGCCTCCAGCTCAGCCCCTGATGAAAACGAGTTTCAATTTGGTTGCCCCCCTGCTCCCTGCCAGGACCCA

25 SerGluProArgValLeuCysCysThrAlaCysLeuSerGluAsnLeuArgAspAspGluAspArgIleCysPro
299 TCGGAGCCCAGAGTTCTCTGCTGCACAGCCTGTCTCTCTGAGAACCTGAGAGATGATGAGGATCGGATCTGTCCT

50 LysCysArgAlaAspAsnLeuHisProValSerProGlySerProLeuThrGlnGluLysValHisSerAspVal
374 AAATGCAGAGCAGACAACCTCCATCCTGTGAGCCCAGGAAGCCCTCTGACTCAGGAGAAGGTTCACTCTGATGTA

75 AlaGluAlaGluIleMetCysProPheAlaGlyValGlyCysSerPheLysGlySerProGlnSerMetGlnGlu
449 GCTGAGGCTGAAATCATGTGCCCCTTTGCAGGTGTTGGCTGTTCCTTCAAGGGGAGCCCACAATCCATGCAGGAG

100 HisGluAlaThrSerGlnSerSerHisLeuTyrLeuLeuLeuAlaValLeuLysGluTrpLysSerSerProGly
524 CATGAGGCTACCTCCCAGTCCTCCCACCTGTACCTGCTGCTGGCGGTCTTAAAGGAGTGGAAATCCTCACCAGGC

125 SerAsnLeuGlySerAlaProMetAlaLeuGluArgAsnLeuSerGluLeuGlnLeuGlnAlaAlaValGluAla
599 TCCAACCTAGGGTCTGCACCCATGGCACTGGAGCGGAACCTGTCAGAGCTGCAGCTTCAGGCAGCTGTGGAAGCG

150 ThrGlyAspLeuGluValAspCysTyrArgAlaProCysCysGluSerGlnGluGluLeuAlaLeuGlnHisLeu
674 ACAGGGGACCTGGAGGTAGACTGCTACCGGGCACCTTGCTGTGAGAGCCAGGAAGAACTGGCCCTGCAGCACTTG

175 ValLysGluLysLeuLeuAlaGlnLeuGluGluLysLeuArgValPheAlaAsnIleValAlaValLeuAsnLys
749 GTGAAGGAGAAGCTGCTGGCTCAGCTGGAGGAGAAGCTGCGTGTGTTTGCAAACATTGTTGCTGTCCTCAACAAG

200 GluValGluAlaSerHisLeuAlaLeuAlaAlaSerIleHisGlnSerGlnLeuAspArgGluHisLeuLeuSer
824 GAAGTGGAGGCTTCCCACCTGGCACTGGCCGCCTCCATCCACCAGAGCCAGTTGGACCGAGAGCACCTCCTGAGC

225 LeuGluGlnArgValValGluLeuGlnGlnThrLeuAlaGlnLysAspGlnValLeuGlyLysLeuGluHisSer
899 TTGGAGCAGAGGGTGGTGGAATTACAGCAAACCCTGGCTCAAAAAGACCAGGTCCTGGGCAAGCTTGAGCACAGT

250 LeuArgLeuMetGluGluAlaSerPheAspGlyThrPheLeuTrpLysIleThrAsnValThrLysArgCysHis
974 CTGCGACTCATGGAGGAGGCATCCTTTGATGGTACTTTCCTGTGGAAGATCACCAATGTCACCAAGCGGTGCCAC

275 GluSerValCysGlyArgThrValSerLeuPheSerProAlaPheTyrThrAlaLysTyrGlyTyrLysLeuCys
1049 GAGTCAGTGTGTGGCCGGACTGTCAGCCTCTTCTCTCCAGCTTTCTACACTGCCAAGTATGGTTACAAGTTGTGC

300 LeuArgLeuTyrLeuAsnGlyAspGlySerGlyLysLysThrHisLeuSerLeuPheIleValIleMetArgGly
1124 CTGCGCTTGTACCTGAACGGGGATGGCTCAGGCAAGAAGACCCACCTGTCCCTCTTCATCGTGATCATGAGAGGA

325 GluTyrAspAlaLeuLeuProTrpProPheArgAsnLysValThrPheMetLeuLeuAspGlnAsnAsnArgGlu
1199 GAATACGATGCTCTCCTGCCCTGGCCTTTCAGGAACAAGGTCACCTTTATGCTACTTGACCAGAACAACCGAGAG

350 HisAlaIleAspAlaPheArgProAspLeuSerSerAlaSerPheGlnArgProGlnSerGluThrAsnValAla
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375 SerGlyCysProLeuPhePheProLeuSerLysLeuGlnSerProLysHisAlaTyrValLysAspAspThrMet
1349 AGCGGCTGCCCGCTCTTCTTCCCCCTCAGCAAGCTGCAGTCACCCAAGCACGCCTACGTCAAAGATGACACAATG

400 PheLeuLysCysIleValAspThrSerAla
1424 TTCCTCAAATGCATTGTGGACACTAGTGCTTAGGGATGGGGGAGGGGGTGTCTCCTGACAGAACCAGCTTAGAC
1499 TGGGGGACTTAGCTAGACAGCCAGGCCCTGCCTGCCCTTGGAGCCCACAGCCCACGACAAGGAGGAGCCAAGGCT
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1799 CTAAGTGGAGGGGCACTCCTACCAGGACATTCTCTCGAGGTCAGGGCATAACTGGAAAAATGCCCCCATCTCTCT
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1949 TCCTATGTTAACAGACACGCAAACAGGTAAACCCAGAAACTGCCCTGGGAAATGCTTTCTGGCTGCATCTGGAGA
2024 TCTTTGATGTTTTTACCGACAAAACAAATAACAAAAGCCTTGAATTGCAAAAAAAAAAAAAAAAA

Figure 10

```
                                                       MetAlaAlaAlaSerValThrSerPro
1
1    GCGCGAAGACCGTTGGGGCTTTGTGGTGTGTGGGGGTTGTAACTCACATGGCTGCAGCCAGTGTGACTTCCCCT

10   GlySerLeuGluLeuLeuGlnProGlyPheSerLysThrLeuLeuGlyThrArgLeuGluAlaLysTyrLeuCys
75   GGCTCCCTAGAACTGCTACAGCCTGGCTTCTCCAAGACCCTCCTGGGGACCAGGTTAGAAGCCAAGTACCTCTGT

35   SerAlaCysLysAsnIleLeuArgArgProPheGlnAlaGlnCysGlyHisArgTyrCysSerPheCysLeuThr
150  TCAGCCTGCAAAAACATCCTGCGGAGGCCTTTCCAGGCCCAGTGTGGGCACCGCTACTGCTCCTTCTGCCTGACC

60   SerIleLeuSerSerGlyProGlnAsnCysAlaAlaCysValTyrGluGlyLeuTyrGluGluGlyIleSerIle
225  AGCATCCTCAGCTCTGGGCCCCAGAACTGTGCTGCCTGTGTCTATGAAGGCCTGTATGAAGAAGGCATTTCTATT

85   LeuGluSerSerSerAlaPheProAspAsnAlaAlaArgArgGluValGluSerLeuProAlaValCysProAsn
300  TTAGAGAGTAGTTCGGCCTTTCCAGATAACGCTGCCCGCAGAGAGGTGGAGAGCCTGCCAGCTGTCTGTCCCAAT

110  AspGlyCysThrTrpLysGlyThrLeuLysGluTyrGluSerCysHisGluGlyLeuCysProPheLeuLeuThr
375  GATGGATGCACTTGGAAGGGGACCTTGAAAGAATACGAGAGCTGCCACGAAGGACTTTGCCCATTCCTGCTGACG

135  GluCysProAlaCysLysGlyLeuValArgLeuSerGluLysGluHisHisThrGluGlnGluCysProLysArg
450  GAGTGTCCTGCATGTAAAGGCCTGGTCCGCCTCAGCGAGAAGGAGCACCACACTGAGCAGGAATGCCCCAAAAGG

160  SerLeuSerCysGlnHisCysArgAlaProCysSerHisValAspLeuGluValHisTyrGluValCysProLys
525  AGCCTGAGCTGCCAGCACTGCAGAGCACCCTGTAGCCACGTGGACCTGGAGGTACACTATGAGGTCTGCCCCAAG

185  PheProLeuThrCysAspGlyCysGlyLysLysLysIleProArgGluThrPheGlnAspHisValArgAlaCys
600  TTTCCCTTAACCTGTGATGGCTGTGGCAAGAAGAAGATCCCTCGGGAGACGTTTCAGGACCATGTTAGAGCATGC

210  SerLysCysArgValLeuCysArgPheHisThrValGlyCysSerGluMetValGluThrGluAsnLeuGlnAsp
675  AGCAAATGCCGGGTTCTCTGCAGATTCCACACCGTTGGCTGTTCAGAGATGGTGGAGACTGAGAACCTGCAGGAT

235  HisGluLeuGlnArgLeuArgGluHisLeuAlaLeuLeuLeuSerSerPheLeuGluAlaGlnAlaSerProGly
750  CATGAGCTGCAGCGGCTACGGGAACACCTAGCCCTACTGCTGAGCTCATTCTTGGAGGCCCAAGCCTCTCCAGGA

260  ThrLeuAsnGlnValGlyProGluLeuLeuGlnArgCysGlnIleLeuGluGlnLysIleAlaThrPheGluAsn
825  ACCTTGAACCAGGTGGGGCCAGAGCTACTCCAGCGGTGCCAGATTTTGGAGCAGAAGATAGCAACCTTTGAGAAC

285  IleValCysValLeuAsnArgGluValGluArgValAlaValThrAlaGluAlaCysSerArgGlnHisArgLeu
900  ATTGTCTGCGTCTTGAACCGTGAAGTAGAGAGGGTAGCAGTGACTGCAGAGGCTTGTAGCCGGCAGCACCGGCTA

310  AspGlnAspLysIleGluAlaLeuSerAsnLysValGlnGlnLeuGluArgSerIleGlyLeuLysAspLeuAla
975  GACCAGGACAAGATTGAGGCCCTGAGTAACAAGGTGCAACAGCTGGAGAGGAGCATCGGCCTCAAGGACCTGGCC

335  MetAlaAspLeuGluGlnLysValSerGluLeuGluValSerThrTyrAspGlyValPheIleTrpLysIleSer
1050 ATGGCTGACCTGGAGCAGAAGGTCTCCGAGTTGGAAGTATCCACCTATGATGGGGTCTTCATCTGGAAGATCTCT

360  AspPheThrArgLysArgGlnGluAlaValAlaGlyArgThrProAlaIlePheSerProAlaPheTyrThrSer
1125 GACTTCACCAGAAAGCGTCAGGAAGCCGTAGCTGGCCGGACACCAGCTATCTTCTCCCCAGCCTTCTACACAAGC

385  ArgTyrGlyTyrLysMetCysLeuArgValTyrLeuAsnGlyAspGlyThrGlyArgGlyThrHisLeuSerLeu
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410  PhePheValValMetLysGlyProAsnAspAlaLeuLeuGlnTrpProPheAsnGlnLysValThrLeuMetLeu
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435  LeuAspHisAsnAsnArgGluHisValIleAspAlaPheArgProAspValThrSerSerSerPheGlnArgPro
1350 CTGGACCATAACAACCGGGAGCATGTGATCGACGCATTCAGGCCCGATGTAACCTCGTCCTCCTTCCAGAGGCCT

460  ValSerAspMetAsnIleAlaSerGlyCysProLeuPheCysProValSerLysMetGluAlaLysAsnSerTyr
1425 GTCAGTGACATGAACATCGCCAGTGGCTGCCCCCTCTTCTGCCCTGTGTCCAAGATGGAGGCCAAGAATTCCTAT

485  ValArgAspAspAlaIlePheIleLysAlaIleValAspLeuThrGlyLeu
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1800 GAAAGCTCCTGCTGTCTCCTCTGTCTGGGGAAGGGAGAGACCTGTAGGTGGGTGCTCAGAAAGGGCCTCTCCAGA
1875 GAGAGTCTCAAGAGCTGCAGCAGGAGCAAAGTGACTGGCCTTCCCCACCCCATCCTTTGGAAAAGAGGTAGCGGC
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2100 ACGACAAAAAAAAAAAAAAAAAA
```

Figure 11

A

Figure 12a

TRAF2	(mouse)	31	KYLCSACKNILRRPFQA	QCGHRYCSFCLTSI	LSS	GPQNCAACVYE
COP1	(*A. thaliana*)	49	DLLCPICMQIIKDAFLT	ACGHSFCYMCIITH	LRN	KSDCPCCSQH
EFP	(human)	10	ELSCSICLEPFKEPVTT	PCGHNFCGSCLNETWA	VQG	SPYLCPQCRAV
RAD-18	(*S. cerevisiae*)	25	LLRCHICKDFLKVPVLT	PCGHTFCSLCIRTH	LNN	QPNCPLCLFE
UVS-2	(*N. crassa*)	31	AFRCHVCKDFYDSPMLT	SCNHTFCSLCIRRC	LSV DSK	CPLCRAT
RAG-1	(human)	290	SISCQICEHILADPVET	NCKHVFCRVCILRC	LKV	MGSYCPSCRYP
SS-A/Ro	(human)	13	EVTCPICLDPFVEPVSI	ECGHSFCQECISQV	GKG	GGSVCAVCRQR
RING1	(human)	16	ELMCPICLDMLKNTMTTKECLHRFCSDCIVTA		LRS	GNKECPTCRKK
RPT-1	(mouse)	12	EVTCPICLELLKEPVSA	DCNHSFCRACITLNYESNRNTDGKGNCPVCRVP		
RFP	(human)	13	ETTCPVCLQYFAEPMML	DCGHNICCACLARCWGTA		ETNVSCPQCRET
c-cbl	(human)	378	FQLCKICAENDKDVKIE	PCGHLMCTSCLTS	WQESEGQ	GSSGCPFCRCE

consensus: ---**C**--**C**-----X11-12-----**C**-**H**--**C**--**C**-----X10-16-----**C**--**C**---

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B

Figure 12b

TRAF2	(mouse)	157	CPKRSLSCQHC RAPCSHVDLEVHYE VC
		182	PKFPLTCDGCGKKKIPRETFQDHVR AC
DG17	(*D. discoideum*)	171	GGFKLVTCDFCKRDDIKKKELETHYK TC
TFIIIA	(*X. laevis*)	189	QD LAVCDVCNRKFRHKDYLRDHQK TH
XLCOF14	(*X. laevis*)	1	TGKYPFICSECGKSFMDKRYLKIHSN VH
XFIN	(*X. laevis*)	1225	TGEKPYTCTVCGKKFIDRSSVVKHSR TH
ZFY1/2	(mouse)	521	RKKFPHICGECGKGFRHPSALKKHIR VH
MFG2	(mouse)	293	SEEKPFECEECGKKFRTARHLVKHQR IH
RAD18	(*S. cerevisiae*)	183	PNEQMAQCPICQQFYPLKALEKTHLD EC
UVS-2	(*N. crassa*)	182	PDDGLVACPICLTRM KEQQVDRHLDTSC

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Tue May 10 17:20:49 1994
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TRAF2   51 H R Y C S F C L T S I L S S G P Q N C A A C V Y E G L Y E E G I S I L E S S S A F P D N A A R R E V

TRAF2  101 E S L P A V C P N D G C T W K G T L K E Y E S C H E G L C P F L L T E C P A C K G L V R L S E K E H
TRAF1    1 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . M A S S S A P D E N E F Q F S C P P A

TRAF2  151 H T E Q E C P K R S L S C Q H C R A P C S H V D L E V H Y E V C P K F P L T C D G C G K K K I P R E
TRAF1   20 P C Q D P S E P R V L C C T A C L S E N L R D D E D R I C P K C R A D N L H P V S P G - S P L T Q E

TRAF2  201 T F Q D H V R A C S K C R V L C R F H T V G C S E M V E T E N L Q D H E L Q R L R E H L A L L L S S
TRAF1   69 K V H S D V - - - A E A E I M C P F A G V G C S F K G S P Q S M Q E H E A T S Q S S H L Y L L L A V

TRAF2  251 F L E A C A S P G T L N Q V G P E L L Q R . . . . . . . . . . . . . . . . . . . . . . . . . . .
TRAF1  116 L K E W K S S P G S N L G S A P M A L E R N L S E L Q L Q A A V E A T G D L E V D C Y R A P C C E S

TRAF2  27? . . . . . . . . . . . . . . . C Q I L E Q K I A T F E N I V C V L N R E V E R V A V T A S A C S R Q H
TRAF1  166 C E E . A . C H L V K E K L L A Q L E E K L R V F A N I V A V L N K E V E A S H L A L A A S I H Q S

TRAF2  306 F . . C C C K I E A L S N K V C Q L E R S I G L K D L A M A D L E Q K V S E L E V S T Y D G V F I W K
TRAF1  216 C . . C P E H L L S L E Q R V V E L C Q T L A Q K D C V L G K L E H S L R L M E E A S F D G T F L W K

TRAF2  356 . S C F . E K P Q E A V A G R T P A I F S P A F Y T S R Y G Y K M C L R V Y L N G D G T G R G T H L
TRAF1  266 . . N . . . F C H E S V C G R T V S L F S P A F Y T A K Y G Y K L C L R L Y L N G D G S G K K T H L

TRAF2  406 S . F F V V M K G P N C A L L Q W P F N Q K V T L M L L D H N N R E H V I D A F R P D V T S S S F Q
TRAF1  316 S . F . . . M P G E Y C A L L P W P F R N K V T F M L L D Q N N R E H A I D A F R P D L S S A S F Q

TRAF2  456 F F . S C M N . A S G C P L F C P V S K M E - A K N S Y V R D D A I F I K A I V D L T G L
TRAF1  366 E F . C S E . N V A S G C P L F F P L S K L Q S P K H A Y V K D D T M F L K C I V D T S A
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Figure 13

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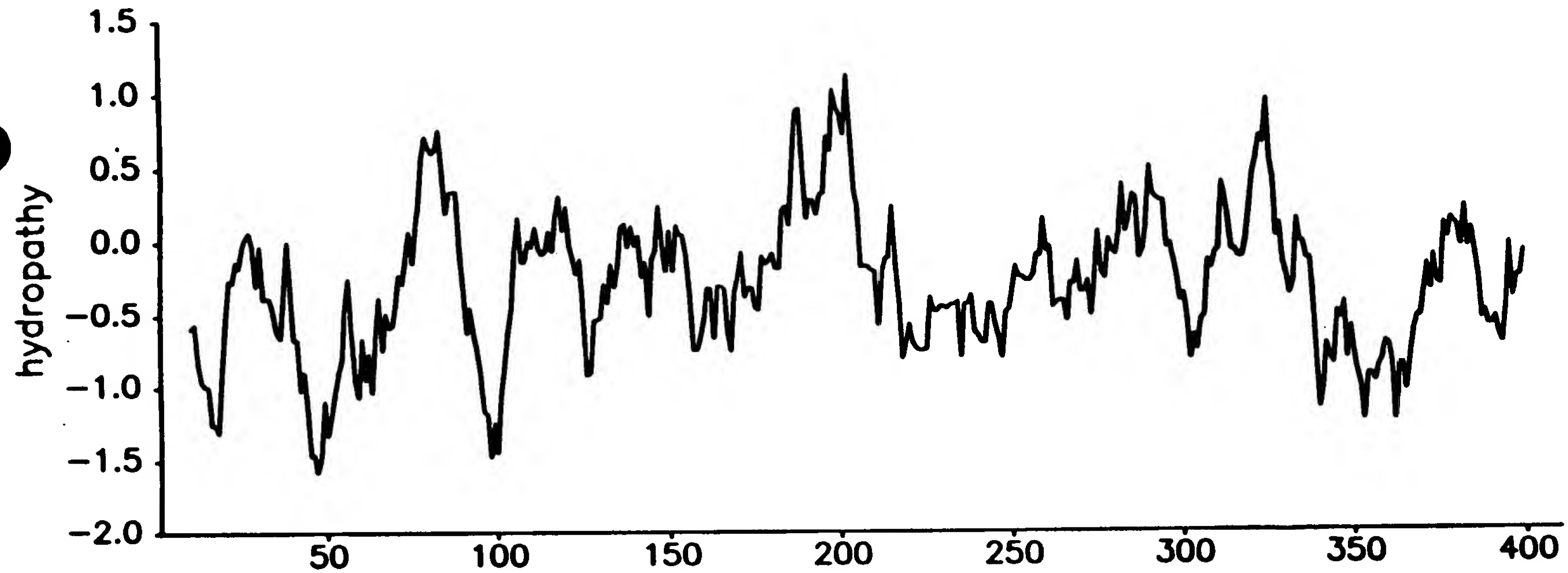

Figure 14a

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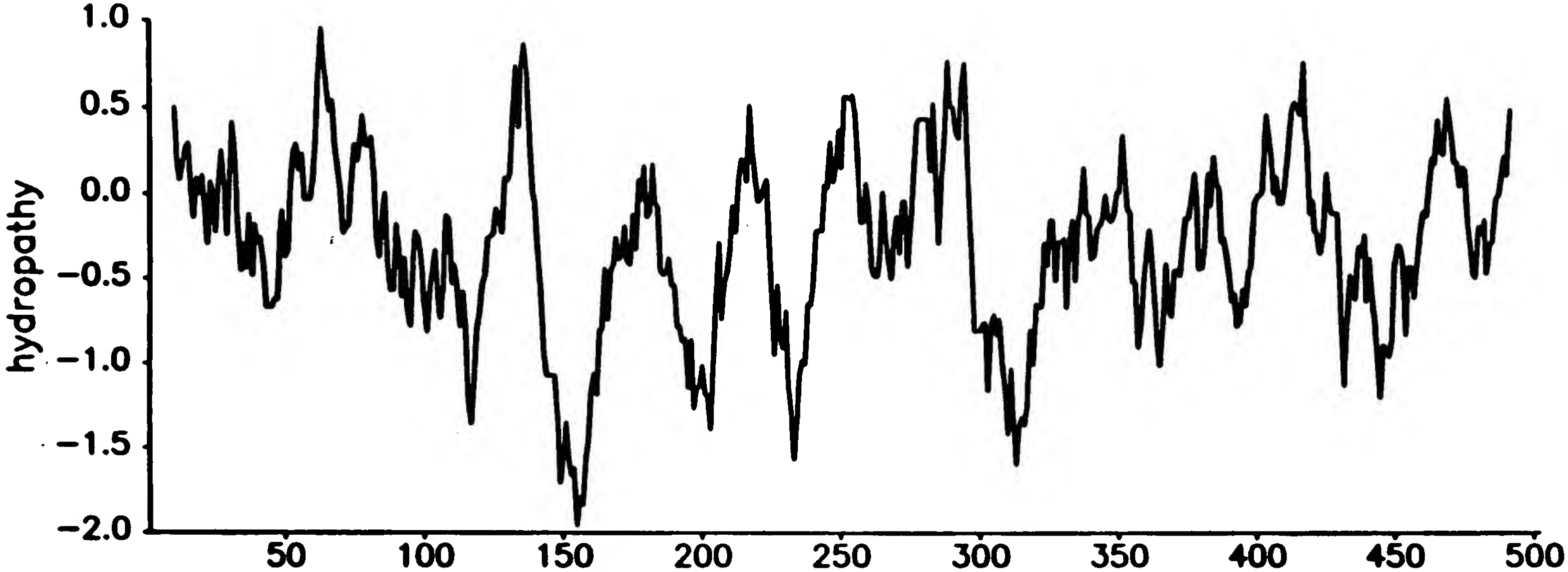

Figure 14b

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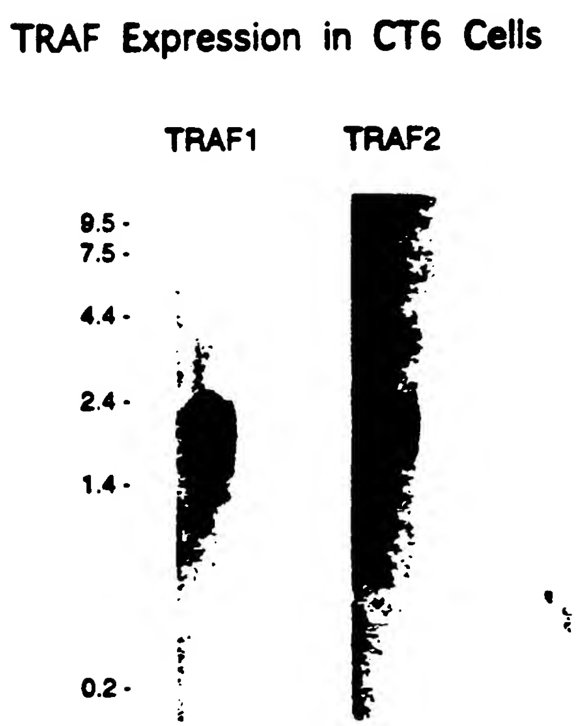
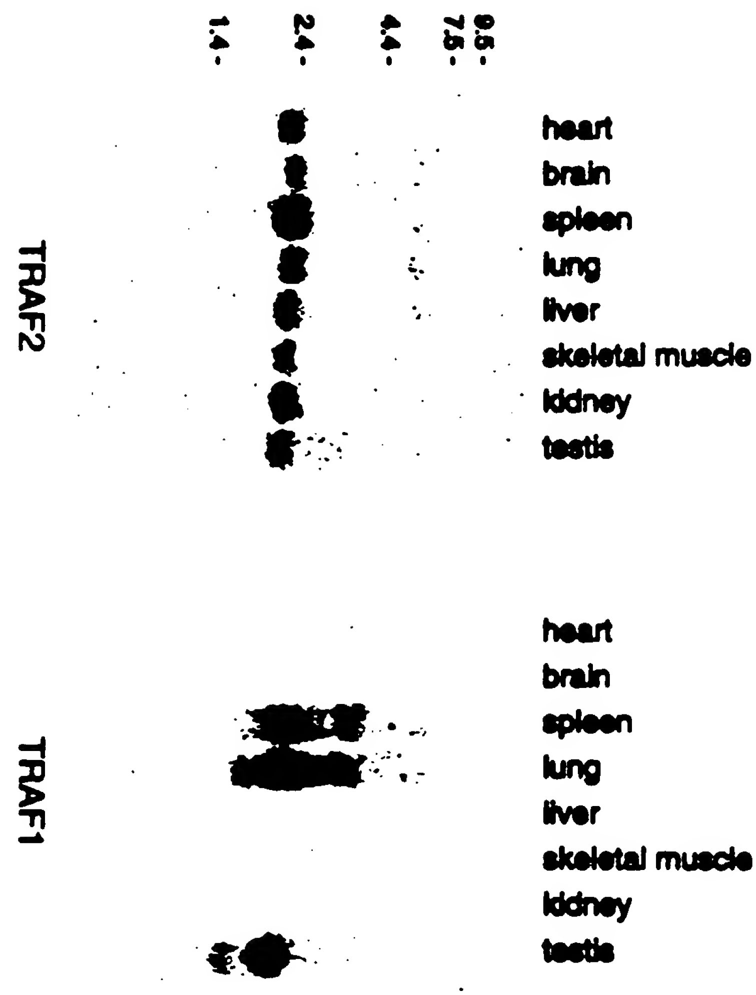


Figure 15a

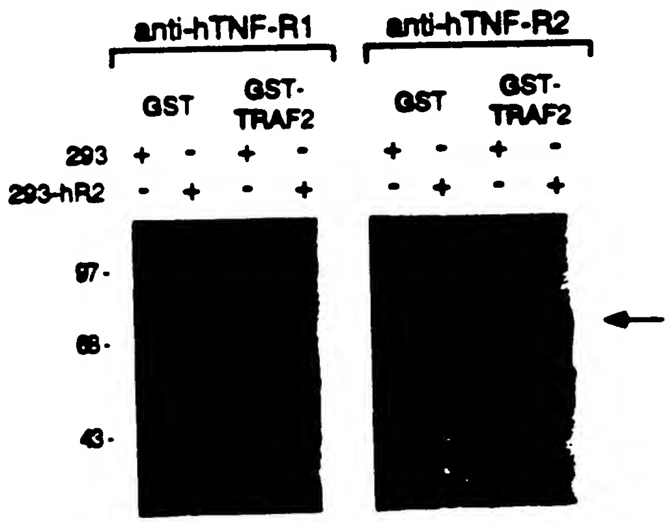
Figure 15b

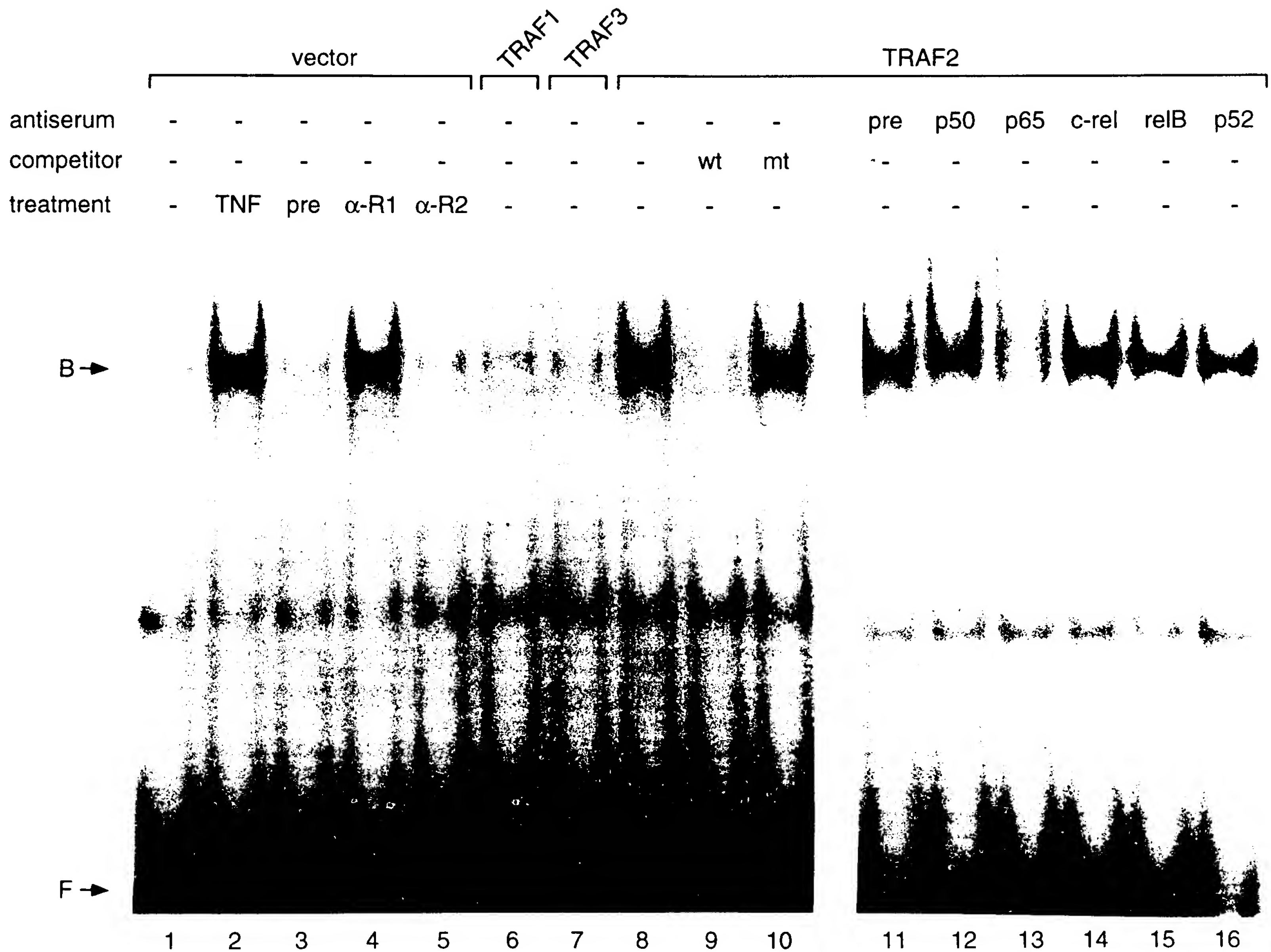


TRAF Expression in Mouse Tissues

Figure 16

A Glutathione-S-Transferase TRAF2 Fusion Protein Coprecipitates the Human TNF-R2 in 293 Cell Extracts

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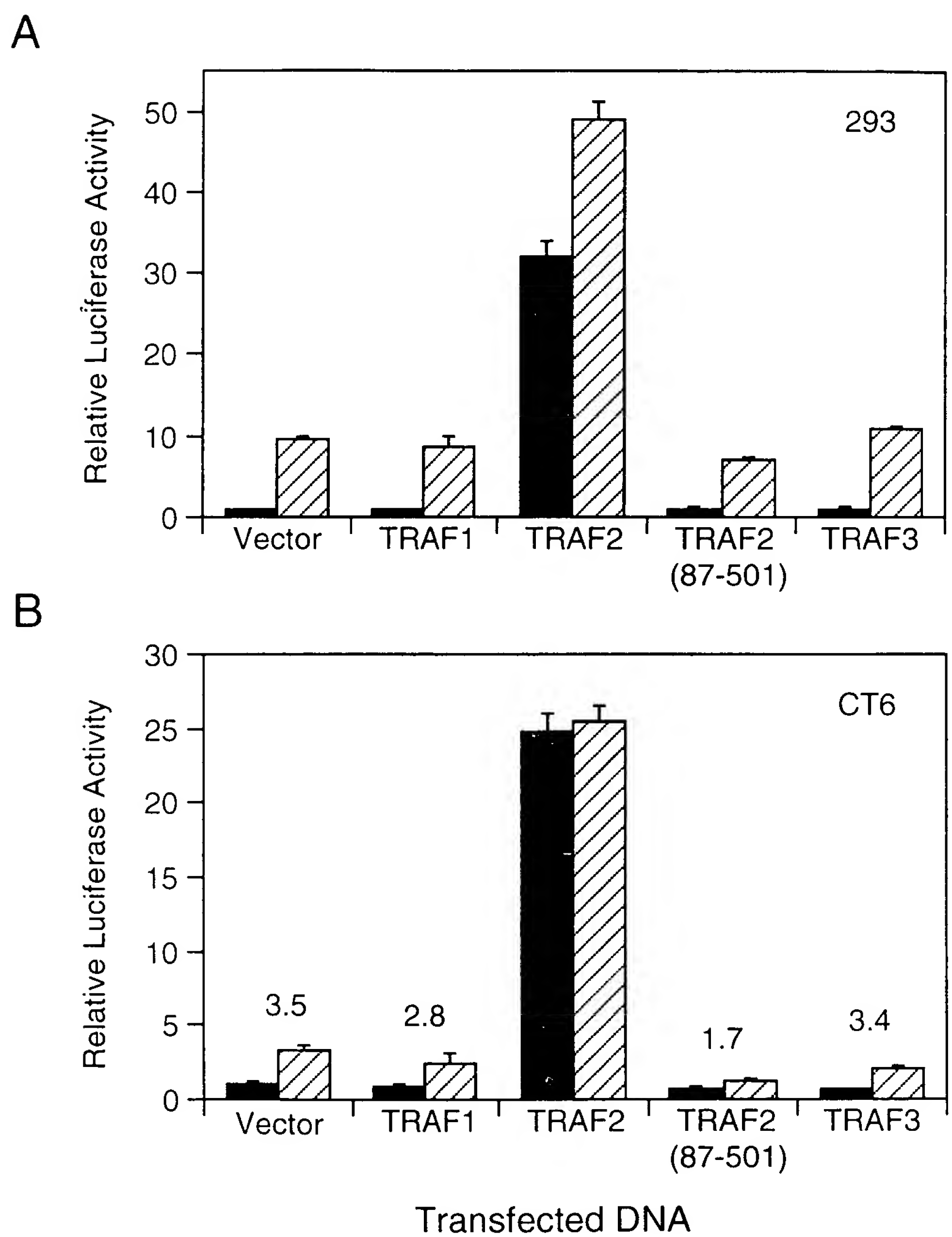
TRAF1
TRAF3
TRAF2
antiserum - - - - - - - - - - pre p50 p65 c-rel relB p52
competitor - - - - - - - - wt mt - - - - - -
treatment - TNF pre α-R1 α-R2 - - - - - - - - - - -
B
F
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

Figure 17

Figure 18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293
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20
30
40
50
Vector
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TRAF2
TRAF2 (87-501)
TRAF3
B
CT6
Relative Luciferase Activity
0
5
10
15
20
25
30
3.5
2.8
1.7
3.4
Vector
TRAF1
TRAF2
TRAF2 (87-501)
TRAF3
Transfected DNA

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Figure 19